

AMENDMENTS TO THE SPECIFICATION

Please replace the text on page 16, line 27 through page 17, line 24 with the following:

- Figure 4 shows that the nine carboxy-terminal amino acids of the M ectodomain constitute a proapoptotic sequence. (A) Amino acid sequence alignments for mutant proteins, the names of which are shown on the right (SEQ ID NO:23 and sections of SEQ ID NO:3). (B) and (C) Transfected HeLa cells were assayed for apoptotic nuclear fragmentation after 25 hours of transfection (B) or for the early stage of apoptosis after 20 hours (C). (B) HeLa cells were stained with Hoescht 33258 and examined for chromatin condensation. C⁹⁵⁻¹¹⁴-tagged EGFP (Control; open box) served as a negative control. The percentages of fusion protein-expressing cells with apoptotic nuclei are indicated. Each experimental point represents the mean \pm the SD of results obtained from three separate chambers. Statistical analysis for fusion proteins were carried out by comparison with the control. (C) The rate of early apoptosis was analyzed by Annexin V binding, as assessed by flow cytometry analysis. Apoptosis in fusion protein-expressing HeLa cells was defined as EGFP-positive cells that bound Annexin V-APC but excluded PI. For each sample, data from 10,000 EGFP-positive cells were collected. The percentages of M¹⁻⁴⁰- and M³²⁻⁴⁰-expressing cells labeled with Annexin V are indicated (square).

- Figure 5 shows that the residues M-34 to M-39 contribute to the death-promoting activity of the M ectodomain. (A) Amino acid sequence (SEQ ID NOS: 24, 25, 23 and 29) alignments of M^{1-40/DEN-2}, M^{1-40/YF.17D} and mutants M^{1-40/DEN-2} (F36) and M^{1-40/YF.17D} (T³⁴, I³⁶, L³⁷, H³⁹). Identical amino acids are indicated (asterisks). The amino acid substitutions are underlined and indicated in bold. (B) After 25 hours of transfection, fusion protein-expressing HeLa cells were stained with Hoechst 33258

and examined for chromatin condensation. The percentages of fusion protein-expressing cells with apoptotic nuclei are indicated. Each experimental point represents the mean \pm the SD of results obtained from three separate chambers. Fusion proteins were compared statistically with C⁹⁵⁻¹¹⁴-tagged EGFP (Control; open box).

- Figure 6 represents the restriction card of plasmid Trip Δ U3 CMV[95-114] EGFP[M₃₂-M₄₀] DEN-2.

- Figure 7 represents the plasmid sequence p[95-114]EGFP[M₁-M₄₀]DEN-2 (I36F) (SEQ ID NOS:30-36).

Please replace the paragraph beginning on page 25, line 17 with the following:

With a view to identifying the minimal sequence of the DEN-2 M ectodomain responsible for the induction of apoptosis, a construct encoding the 9 carboxy-terminal amino acids located at positions 32 to 40 fused to EGFP was engineered (Fig. 4A). The Inventors have investigated M^{32-40/DEN-2}-mediated cell death by flow cytometry, using the Annexin V affinity assay, which detects phosphatidylserine (PS) translocated to the outer layer of the cell membrane. The exposure of membrane PS is an early indicator of apoptosis. The fusion proteins C⁹⁵⁻¹¹⁴-EGFP-M^{1-30/DEN-2} and C⁹⁵⁻¹¹⁴-EGFP-M^{1-40/DEN-2} were used as negative and positive controls, respectively. In 3 independent experiments, the transfected HeLa cells producing C⁹⁵⁻¹¹⁴-EGFP-M^{32-40/DEN-2} displayed significantly higher fraction of EGFP-positive cells labeled with Annexin V-APC than did cells producing C⁹⁵⁻¹¹⁴-EGFP-M^{1-40/DEN-2} (Fig. 4C, squares). Thus, residues ³²IETWALRHP⁴⁰ (SEQ ID NO:32-40 of SEQ ID NO:23) are responsible for the death-promoting activity of DEN-2 ecto-M. HeLa cells producing C⁹⁵⁻¹¹⁴-tagged EGFP and C⁹⁵⁻¹¹⁴-EGFP-M^{1-30/DEN-2} also contained a subpopulation of

Annexin V-labeled cells (Fig. 4C). It is likely that overproduction of EGFP has cytotoxic effects.

After page 33 (Abstract), please insert the attached sequence listing.

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